[4] *On the Mathematical Foundations of Theoretical Statistics, Phil. Trans., London,* **A222,** 315.

[5] *The Goodness of Fit of Regression Formulae and the Distribution of Regression Coefficients, J. R. Stat. Soc., London,* **85,** 597–612.

[6] *The Distribution of the Partial Correlation Coefficient, Metron,* **3,** 329 (1924).

[7] *The Influence of Rainfall on the Yield of Wheat at Rothamsted, Phil. Trans., London,* **B213,** 91, 93 (1923).

[8] This important expression for the volume element has been used in lectures by Professors O. Veblen and L. P. Eisenhart. I do not find it in any of the treatises on Calculus, Analysis or Differential Geometry, save for the special case in which the manifold of integration is a surface. It may readily be proved by showing first that (17) is a relative invariant under arbitrary transformations of the parameters; and second, that if the parameters of the hypersurface are orthogonal at a point, (17) becomes at this point the simple expression for the volume element in cartesian coördinates.

THE LIFE HISTORY OF A MICRO-PARASITE ISOLATED FROM CARCINOMATOUS GROWTHS

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The recent awakening of active interest in the possible parasitic origin of carcinoma has prompted the publication of this preliminary report upon an investigation in this direction, which is still in progress.

It should be mentioned in advance that Young,[1] Louden and McCormack[2] and Scott[3] (who present also the unpublished work of Glover), and Nuzum[4] have all described an organism possessing certain salient features in common with that here described. Young traces the presence of an organism from a "plasm" stage through several organized, "morphologically recognizable" stages. Louden and McCormack have isolated an organism with a very complex life history, which results are analogous to those of men like Löhnis and Smith and others who worked on life cycles of bacteria. Nuzum's extensive work on the micrococcus isolated by him may represent one life stage of the same organism which has attained a certain stabilization of form. The present research was, however, entirely independent of previous ones; indeed the organism was obtained by us before we knew of the publications here referred to. The results of this research are thus in some measure a confirmation and in greater measure an extension of the work of these investigators.

The organism described in this paper exhibits a complex life history, and it possesses morphological characteristics in its parasitic environ-

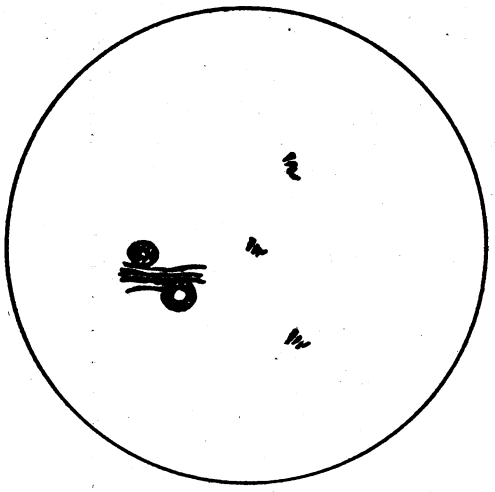

FIGURE 1

Showing spirilla in tissues and fluid which by special methods stain to a deep violet-black color; showing also distinct globules.

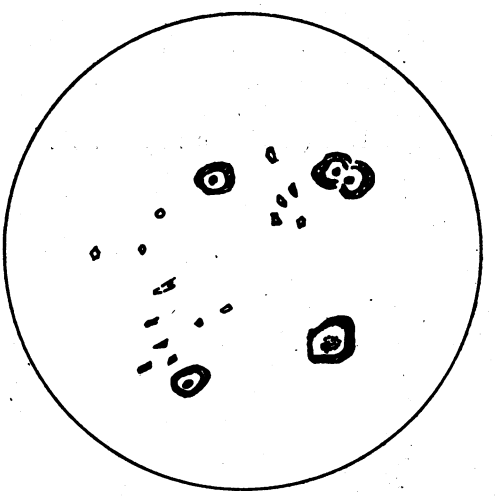

FIGURE 2

Showing granules and sporulating bodies as first seen in aerobic tissue cultures. Both take carbol fuchsin stain only slightly.

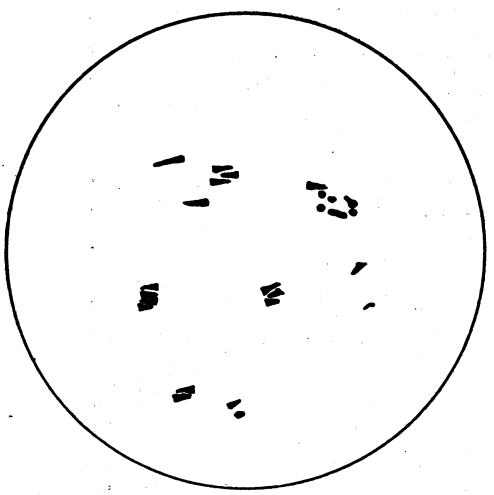

FIGURE 3

Showing minute bacilli with tapering ends as first seen after 24 hours' incubation of granules and sporing bodies.

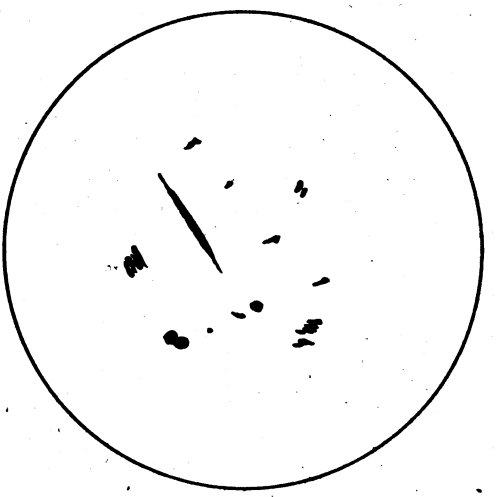

FIGURE 4

Showing a 48-hour culture on solid medium. There is a decided increase in size. All stages are seen here before stabilization has taken place due to acclimatization to saprophytic environment.

ment which differ greatly from those which it has in its saprophytic environment. In the fluid from the tissue the organism is almost invisible, and passes through filters, but can be detected by the special methods described below. It presents the appearance of a delicate, loosely coiled spirillum (as shown in Figs. 1 and 5). When a piece of carcinomatous tissue, obtained under aseptic conditions, is implanted into deep tubes of a specially prepared solid medium, there appears, after incubation for three to five days, minute white pinhead outgrowths. Microscopically these consist of delicate threads showing here and there globules or spheres with concentric rings (as seen in the lower left quadrant of Fig. 1). Grown aerobically, a spreading, transparent, glistening growth surrounds the tissue which sometimes appears after only 24 hours' incubation. Granules which do not stain readily and globules with concentric rings (Fig. 2) can be seen in great profusion. These granules soon give place to minute bacilli which gradually increase in size and vary greatly in shape and formation (Fig. 3). A peculiarity of these bacilli is their close "fence post" arrangement, which, together with the fact that they taper at one end, makes it difficult to distinguish them from the coiled spirilla.

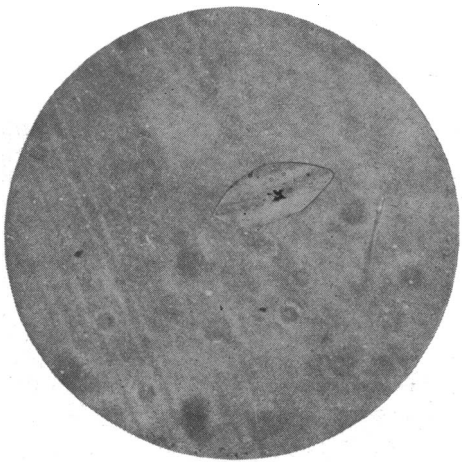

FIGURE 5
Showing coiled spirilla in the fluid × 1950.

This interpretation of the life history is based on a close study of single-cell cultures obtained by the method of Orskov.[5] It is represented more fully by the drawings (Figs. 1 to 4), and by the photographs (Figs. 5 to 8). These are described by the legends below the respective figures.

As will be seen, the life history includes rods of varying sizes, formations and shapes: threadlike forms with tapering ends; cocci, very minute or very large; and large sporing bodies, indicating endosporulation. A pure culture of each stage can be obtained on solid media when conditions, i.e., media and aqueous tension, are held constant; but this is difficult in liquid media.

This organism shows preference for temperatures of 37° to 41°C., while most parasitic fungi are known to grow best at from 30° to 35°C. It can be made to grow under either aerobic or anaerobic conditions.

On potato slants, as well as on solid media, the minute bacillus appeared

as a deep sulfur-yellow growth and gradually lost its color as it approached the coccus stage, in which it was white.

Russell's double sugar medium became pink after 24 to 48 hours' incubation, indicating acid formation. When either the minute bacillus or the coccus was inoculated into sugar broth, gas formation never resulted, but acid was always produced. The broths used in separate experiments contained 1% and 2% of lactose, glucose, levulose or sucrose. The acid produced was lactic acid, all tubes giving a positive test for this acid.

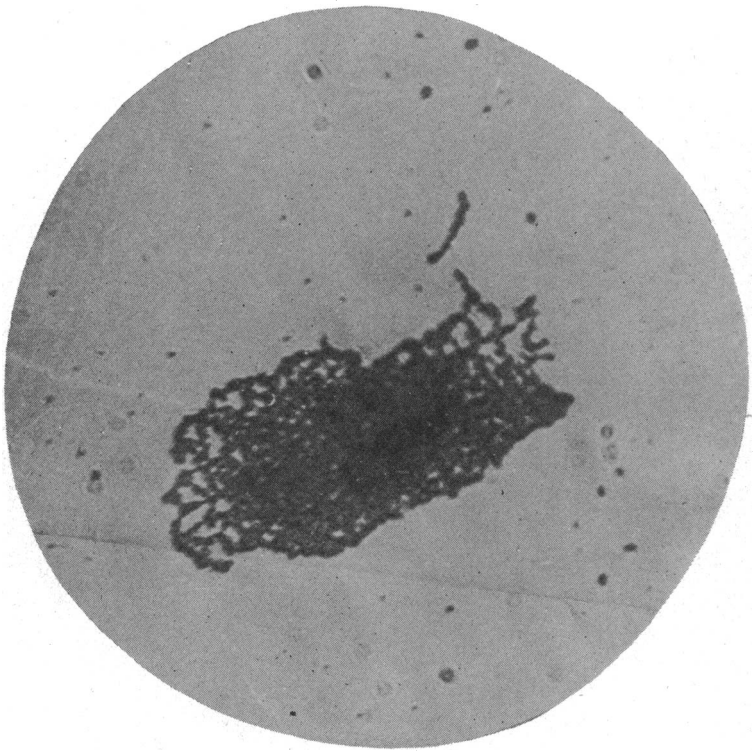

FIGURE 6
Showing minute cocco-bacillus × 1950.

This is significant in the light of the well-known fact that carcinomatous tissue produces large amounts of lactic acid when cultured in sugar media.

By using the following methods, the cancer extract and tissue showed distinctly the spirillary body, which is so extremely delicate as to approach ultra-microscopic size. Fluids removed from cancer tissue were placed in a buffered formaldehyde solution adjusted to a pH of about 8, and, in case of the best results, were kept there for several months; they were then smeared on glass slides and stained by intensive steaming methods with

Wright's stain which had been fortified with sodium carbonate; or the fluids were filtered through a Berkfeldt filter and a gentian-violet–dichromate method of staining was employed, based on the staining principles outlined previously by Stearn and Stearn.[6] This latter method was also used for the staining of the paraffin sections of the tissue grown in deep agar tubes.

The Gram character of the organism varied with the different stages in the life cycle. There was an observable tendency for the most part toward Gram positiveness.

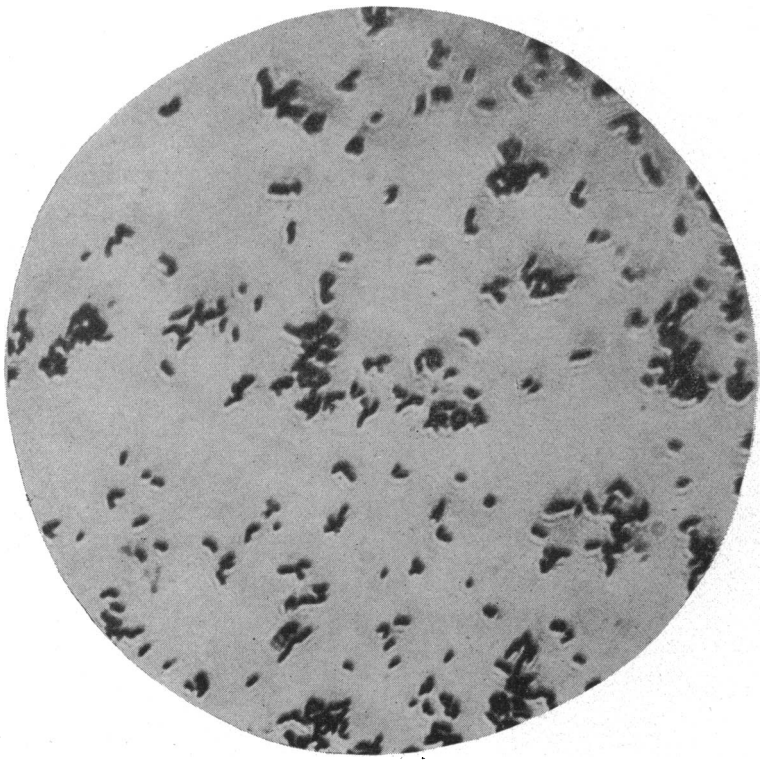

FIGURE 7

Showing rods with tapering ends and some with hooked ends; also the coccus form (× 1950); illustrating the great variation from a minute bacillus to one of considerable size.

One of the authors (B. F. Sturdivant), who made an examination of nearly five hundred specimens of non-carcinomatous tissue removed at operations, was unable to demonstrate the presence of this peculiar spirillary organism in them, but upon examination of carcinomatous tissues he detected it. This suggests a possible etiological relationship and the work of the investigators cited above points also in this direction.

In this connection it is well to keep in mind the work of Ferran,[7] which shows that there are three distinct stages in the life history of the tubercle bacillus; also the work of Much[8] on the super-virulence of the Gram-positive granules which compose the second stage of the life cycle of this bacillus. This work and that of others on other organisms lead to the belief that much of the confusion which prevails regarding the various organisms which have from time to time been isolated from cancer tissues arises

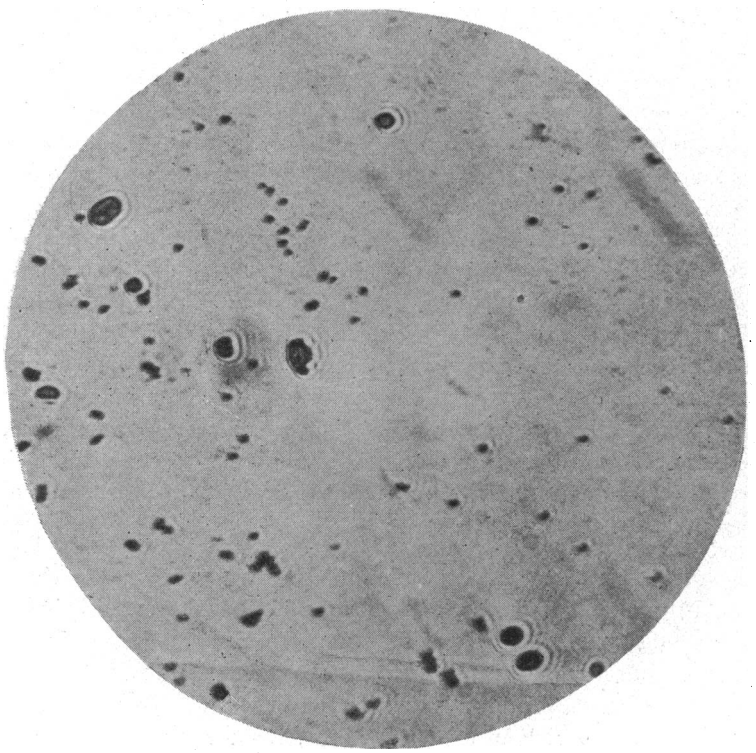

FIGURE 8
Showing the coccus form and the large globules indicating endo-sporulation (× 1950).

from the fact that an organism may assume various forms, including the ultra-microscopic and the nearly microscopic and that in only one of these forms it may be capable of disturbing the cell equilibrium of the host. There may also be mentioned the work of Hort,[9] who thinks that the meningococcus, as we know it, is not the primary cause of epidemic cerebrospinal fever. For he was unable to produce the disease in monkeys by injection of laboratory cultures of the organism; but showed that the cerebrospinal fluids of patients contains a filterable virus which would produce

continuous fever and cause death in monkeys; and the inoculation of this filtrate into culture media yielded the ordinary form of meningococcus and also other forms encountered in this disease. He believes that the virus and the meningococcus are different stages in the life cycle of the same organism.

With respect to the chemical substances produced by the organism it should be mentioned that orthorhombic crystals appeared within its colonies under certain conditions. Moreover, the anaerobic colonies showed a tendency to adhere to the plate on which they are cultured as sharp crystalline masses; and it was evident that the organism had encased itself in this deposit. When an agar plate on which isolated colonies had grown was completely dried, the final appearance of the plate resembled that of clusters of white crystals. These crystals are readily produced on solid media containing extract of muscle tissue.

It is possible that this crystalline substance is related to the pathological activity of the organism, which may be innocuous under most conditions, but under certain conditions may produce metabolic products which stimulate cell proliferation. This would accord with the recently published findings of Gye on chicken sarcoma produced by the Rous virus. He states that the virus alone is unable to bring about the malignant transformation of a cell, and concludes that a specific factor, "probably a chemical substance," ruptures the cell defenses and enables the virus to infect. It should be pointed out also that in all his experiments there was a trace of silica present in the inocula.

Summary.—The essential features of this investigation are the invariable identification of a minute microörganism in true human carcinomatous growths, its subsequent cultivation therefrom and the tracing of the complicated life history which it appears to have. Cultures of either the tissue or the extract from such growths yield the organism; while the culturing of growths, which are non-malignant, has never yielded an organism of the form recognized in malignant growths.

Of especial interest is also the fact that carcinomatous tissue which has been incubated at 37°C. in deep tubes of a solid medium for a month or longer, when removed, sectioned in paraffin and stained by a special gentian-violet–dichromate technique, was found to contain great numbers of these minute spirillary bacilli—a fact which may prove of significance in the diagnosis of cancerous growths.

The bacteriological investigation is being continued. The definite classification of the organism according to the requirements of the Society of American Bacteriologists will be worked out as soon as possible by isolating several hundred strains from different sources, so as to corroborate

the findings here reported. The possible relationship of this organism to the production of carcinomatous growths is also being studied by means of animal experimentation. The metabolic by-products produced by the organism at various stages of its life history are being investigated and their effect on normal cell development will be tested.

[1] Young, *Edin. Med. Jour.*, **27**, 212 (1921); **28**, 233 (1922); **29**, 110 (1922); **31**, 163 (1924); *Brit. Med. Jour.*, Feb., 1925.

[2] Louden and McCormack, *Canadian Lancet and Pract.*, Jan., 1925, and May, 1925.

[3] Scott, *Northwest Med.*, April, 1925 and May, 1925.

[4] Nuzum, *Surg. Gyn. and Obs.*, **40**, 343 (1925).

[5] Orskov, *Jour. Bact.*, **7**, 537 (1922).

[6] Stearn and Stearn, *Jour. Bact.*, **9**, 463 (1924).

[7] Ferran, *Travaux sur la Nouvelle Bacteriologic de la Tuberculose*, Barcelone Imp., La Rensixensa, 1913, 13.

[8] Much, *Beit. z. Klinik d. Tuberculose*, **8**, 85 and 357 (1907); **9**, 67 (1908); supplement, **6** (1913).

[9] Hort, *Brit. Med. Jour.*, Mar. 27, 1915 and April 24, 1915.

[10] Gye, *Lancet*, **209**, 109 (July 18, 1925).